PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07D 409/14 — A1

(11) International Publication Number: **WO 97/28160**

(43) International Publication Date: 7 August 1997 (07.08.97)

(21) International Application Number: PCT/US97/00955

(22) International Filing Date: 31 January 1997 (31.01.97)

(30) Priority Data:
60/010,992 — 1 February 1996 (01.02.96) — US

(71) Applicant *(for all designated States except US)*: SMITHKLINE BEECHAM CORPORATION [US/US]; Corporate Intellectual Property, UW2220, 709 Swedeland Road, P.O. Box 1539, King of Prussia, PA 19406-0939 (US).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: ELLIOTT, John, Duncan [GB/US]; 723 Old Eagle School Road, Wayne, PA 19087 (US).

(74) Agents: HALL, Linda, E. et al.; SmithKline Beecham Corporation, Corporate Intellectual Property, UW2220, 709 Swedeland Road, P.O. Box 1539, King of Prussia, PA 19406-0939 (US).

(81) Designated States: JP, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: ENDOTHELIN RECEPTOR ANTAGONISTS

(57) Abstract

Novel isooxazoles, oxazoles, thiazoles, isothiazoles and imidazoles, pharmaceutical compositions containing these compounds and their use as endothelin receptor antagonists are described.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	**GB**	United Kingdom	**MW**	Malawi
AT	Austria	**GE**	Georgia	**MX**	Mexico
AU	Australia	**GN**	Guinea	**NE**	Niger
BB	Barbados	**GR**	Greece	**NL**	Netherlands
BE	Belgium	**HU**	Hungary	**NO**	Norway
BF	Burkina Faso	**IE**	Ireland	**NZ**	New Zealand
BG	Bulgaria	**IT**	Italy	**PL**	Poland
BJ	Benin	**JP**	Japan	**PT**	Portugal
BR	Brazil	**KE**	Kenya	**RO**	Romania
BY	Belarus	**KG**	Kyrgystan	**RU**	Russian Federation
CA	Canada	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CF	Central African Republic			**SE**	Sweden
CG	Congo	**KR**	Republic of Korea	**SG**	Singapore
CH	Switzerland	**KZ**	Kazakhstan	**SI**	Slovenia
CI	Côte d'Ivoire	**LI**	Liechtenstein	**SK**	Slovakia
CM	Cameroon	**LK**	Sri Lanka	**SN**	Senegal
CN	China	**LR**	Liberia	**SZ**	Swaziland
CS	Czechoslovakia	**LT**	Lithuania	**TD**	Chad
CZ	Czech Republic	**LU**	Luxembourg	**TG**	Togo
DE	Germany	**LV**	Latvia	**TJ**	Tajikistan
DK	Denmark	**MC**	Monaco	**TT**	Trinidad and Tobago
EE	Estonia	**MD**	Republic of Moldova	**UA**	Ukraine
ES	Spain	**MG**	Madagascar	**UG**	Uganda
FI	Finland	**ML**	Mali	**US**	United States of America
FR	France	**MN**	Mongolia	**UZ**	Uzbekistan
GA	Gabon	**MR**	Mauritania	**VN**	Viet Nam

ENDOTHELIN RECEPTOR ANTAGONISTS

FIELD OF INVENTION

The present invention relates to isooxazoles, oxazoles, thiazoles, isothiazoles and imidazoles, pharmaceutical compositions containing these compounds and their use as endothelin receptor antagonists.

Endothelin (ET) is a highly potent vasoconstrictor peptide synthesized and released by the vascular endothelium. Endothelin exists as three isoforms, ET-1, ET-2 and ET-3. [Unless otherwise stated "endothelin" shall mean any or all of the isoforms of endothelin]. Endothelin has profound effects on the cardiovascular system, and in particular, the coronary, renal and cerebral circulation. Elevated or abnormal release of endothelin is associated with smooth muscle contraction which is involved in the pathogenesis of cardiovascular, cerebrovascular, respiratory and renal pathophysiology. Elevated levels of endothelin have been reported in plasma from patients with essential hypertension, acute myocardial infarction, subarachnoid hemorrhage, atherosclerosis, and patients with uraemia undergoing dialysis.

In vivo, endothelin has pronounced effects on blood pressure and cardiac output. An intravenous bolus injection of ET (0.1 to 3 nmol/kg) in rats causes a transient, dose-related depressor response (lasting 0.5 to 2 minutes) followed by a sustained, dose-dependent rise in arterial blood pressure which can remain elevated for 2 to 3 hours following dosing. Doses above 3 nmol/kg in a rat often prove fatal.

Endothelin appears to produce a preferential effect in the renal vascular bed. It produces a marked, long-lasting decrease in renal blood flow, accompanied by a significant decrease in GFR, urine volume, urinary sodium and potassium excretion. Endothelin produces a sustained antinatriuretic effect, despite significant elevations in atrial natriuretic peptide. Endothelin also stimulates plasma renin activity. These findings suggest that ET is involved in the regulation of renal function and is involved in a variety of renal disorders including acute renal failure, cyclosporine nephrotoxicity, radio contrast induced renal failure and chronic renal failure.

Studies have shown that in vivo, the cerebral vasculature is highly sensitive to both the vasodilator and vasoconstrictor effects of endothelin. Therefore, ET may be an important mediator of cerebral vasospasm, a frequent and often fatal consequence of subarachnoid hemorrhage.

ET also exhibits direct central nervous system effects such as severe apnea and ischemic lesions which suggests that ET may contribute to the development of cerebral infarcts and neuronal death.

ET has also been implicated in myocardial ischemia (Nichols et al. Br. J. Pharm. 99: 597-601, 1989 and Clozel and Clozel, Circ. Res., 65: 1193-1200, 1989) coronary vasospasm (Fukuda et al., Eur. J. Pharm. 165: 301-304, 1989 and Lüscher, Circ. 83: 701, 1991) heart failure, proliferation of vascular smooth muscle cells, (Takagi, Biochem & Biophys. Res. Commun.; 168: 537-543, 1990, Bobek et al., Am. J. Physiol. 258:408-C415, 1990) and atherosclerosis, (Nakaki et al., Biochem. & Biophys. Res. Commun. 158: 880-881, 1989, and Lerman et al., New Eng. J. of Med. 325: 997-1001, 1991). Increased levels of endothelin have been shown after coronary balloon angioplasty (Kadel et al., No. 2491 Circ. 82: 627, 1990).

Further, endothelin has been found to be a potent constrictor of isolated mammalian airway tissue including human bronchus (Uchida et al., Eur J. of Pharm. 154: 227-228 1988, LaGente, Clin. Exp. Allergy 20: 343-348, 1990; and Springall et al., Lancet, 337: 697-701, 1991). Endothelin may play a role in the pathogenesis of interstitial pulmonary fibrosis and associated pulmonary hypertension, Glard et al., Third International Conference on Endothelin, 1993, p. 34 and ARDS (Adult Respiratory Distress Syndrome), Sanai et al., Supra, p. 112.

Endothelin has been associated with the induction of hemorrhagic and necrotic damage in the gastric mucosa (Whittle et al., Br. J. Pharm. 95: 1011-1013, 1988); Raynaud's phenomenon, Cinniniello et al., Lancet 337: 114-115, 1991); Crohn's Disease and ulcerative colitis, Munch et al., Lancet, Vol. 339, p. 381; Migraine (Edmeads, Headache, Feb. 1991 p 127); Sepsis (Weitzberg et al., Circ. Shock 33: 222-227, 1991; Pittet et al., Ann. Surg. 213: 262-264, 1991), Cyclosporin-induced renal failure or hypertension (Eur. J. Pharmacol., 180: 191-192, 1990, Kidney Int. 37: 1487-1491, 1990) and endotoxin shock and other

endotoxin induced diseases (Biochem. Biophys. Res. Commun., 161: 1220-1227, 1989, Acta Physiol. Scand. 137: 317-318, 1989) and inflammatory skin diseases. (Clin Res. 41:451 and 484, 1993).

Endothelin has also been implicated in preclampsia of pregnancy. Clark et al., Am. J. Obstet. Gynecol. March 1992, p. 962-968; Kamor et al., N. Eng. J. of Med., Nov 22, 1990, p. 1486-1487; Dekker et al., Eur J. Ob. and Gyn. and Rep. Bio. 40 (1991) 215-220; Schiff et al., Am. J. Ostet. Gynecol. Feb 1992, p. 624-628; diabetes mellitus, Takahashi et al., Diabetologia (1990) 33:306-310; and acute vascular rejection following kidney transplant, Watschinger et al., Transplantation Vol. 52, No. 4, pp. 743-746.

Endothelin stimulates both bone resorption and anabolism and may have a role in the coupling of bone remodeling. Tatrai et al. Endocrinology, Vol. 131, p. 603-607.

Endothelin has been reported to stimulate the transport of sperm in the uterine cavity, Casey et al., J. Clin. Endo and Metabolism, Vol. 74, No. 1, p. 223-225, therefore endothelin antagonists may be useful as male contraceptives. Endothelin modulates the ovarian/menstrual cycle, Kenegsberg, J. of Clin. Endo. and Met., Vol. 74, No. 1, p. 12, and may also play a role in the regulation of penile vascular tone in man, Lau et al., Asia Pacific J. of Pharm., 1991, 6:287-292 and Tejada et al., J. Amer. Physio. Soc. 1991, H1078-H1085. Endothelin also mediates a potent contraction of human prostatic smooth muscle, Langenstroer et al., J. Urology, Vol. 149, p. 495-499.

Thus, endothelin receptor antagonists would offer a unique approach toward the pharmacotherapy of hypertension, renal failure, ischemia induced renal failure, sepsis-endotoxin induced renal failure, prophylaxis and/or treatment of radio-contrast induced renal failure, acute and chronic cyclosporin induced renal failure, cerebrovascular disease, myocardial ischemia, angina, heart failure, asthma, pulmonary hypertension, pulmonary hypertension secondary to intrinsic pulmonary disease, atherosclerosis, Raynaud's phenomenon, ulcers, sepsis, migraine, glaucoma, endotoxin shock, endotoxin induced multiple organ failure or disseminated intravascular coagulation, cyclosporin-induced renal failure and as an

adjunct in angioplasty for prevention of restenosis, diabetes, preclampsia of pregnancy, bone remodeling, kidney transplant, male contraceptives, infertility and priaprism and benign prostatic hypertrophy.

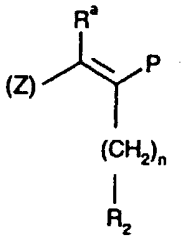
SUMMARY OF THE INVENTION

This invention comprises compounds represented by Formula (I) and pharmaceutical compositions containing these compounds, and their use as endothelin receptor antagonists which are useful in the treatment of a variety of cardiovascular and renal diseases including but not limited to: hypertension, acute and chronic renal failure, cyclosporine induced nephrotoxicity, benign prostatic hypertrophy, pulmonary hypertension, migraine, stroke, cerebrovascular vasospasm, myocardial ischemia, angina, heart failure, atherosclerosis, and as an adjunct in angioplasty for prevention of restenosis.

This invention further constitutes a method for antagonizing endothelin receptors in an animal, including humans, which comprises administering to an animal in need thereof an effective amount of a compound of Formula (I).

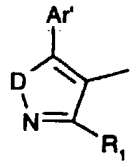
DETAILED DESCRIPTION OF THE INVENTION

The compounds of this invention are represented by structural Formula (I):

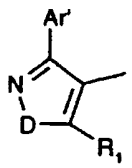


(I)

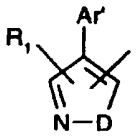
wherein Z is



(d)

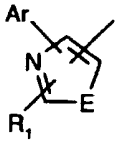


(e)



(f)

or



(g)

D is O or S;

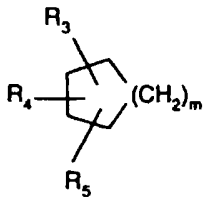
E is O, S or NR_{15};

P is tetrazol-5-yl, CO_2R_6 or $C(O)N(R_6)S(O)_qR_{10}$;

R^a is hydrogen or C_{1-6}alkyl;

R_1 is independently hydrogen, Ar or C_{1-6}alkyl;

R_2 is Ar, C_{1-8}alkyl, $C(O)R_{14}$ or



(c)

R_3 and R_5 are independently R_{13} OH, C_{1-8}alkoxy, $S(O)_qR_{11}$, $N(R_6)_2$, Br, F, I, Cl, CF_3, $NHCOR_6$ $R_{13}CO_2R_7$, -X-R9-Y or $-X(CH_2)_nR_8$ wherein each methylene group within $-X(CH_2)_nR_8$ may be unsubstituted or substituted by one or two $-(CH_2)_nAr$ groups;

R_4 is independently R_{11}, OH, C_{1-5}alkoxy, $S(O)_qR_{11}$, $N(R_6)_2$, Br, F, I, Cl or $NHCOR_6$, wherein the C_{1-5}alkoxy may be unsubstituted or substituted by OH, methoxy or halogen;

R_6 is independently hydrogen or C_{1-8}alkyl;

R_7 is independently hydrogen, C_{1-10}alkyl, C_{2-10}alkenyl or C_{2-8}alkynyl, all of which may be unsubstituted or substituted by one or more OH, $N(R_6)_2$, CO_2R_{12}, halogen or XC_{1-10}alkyl; or R_7 is $(CH_2)_nAr$;

R_8 is independently R_{11}, CO_2R_7, $CO_2C(R_{11})_2O(CO)XR_7$, $PO_3(R_7)_2$, $SO_2NR_7R_{11}$, $NR_7SO_2R_{11}$, $CONR_7SO_2R_{11}$, SO_3R_7, SO_2R_7, $P(O)(OR_7)R_7$, CN, $CO_2(CH_2)_mC(O)N(R_6)_2$, $C(R_{11})_2N(R_7)_2$, $C(O)N(R_6)_2$, $NR_7C(O)NR_7SO_2R_{11}$, tetrazole or OR_6;

R_9 is independently a bond, C_{1-10}alkylene, C_{1-10}alkenylene, C_{1-10}alkylidene, C_{1-10}alkynylene, all of which may be linear or branched, or phenylene, all of which may be unsubstituted or substituted by one of more OH, $N(R_6)_2$, COOH or halogen;

R_{10} is independently C_{1-10}alkyl, $N(R_6)_2$ or Ar;

R_{11} is independently hydrogen, Ar, C_{1-8}alkyl, C_{2-8}alkenyl, C_{2-8}alkynyl, all of which may be unsubstituted or substituted by one or more OH, CH_2OH, $N(R_6)_2$ or halogen;

R_{12} is independently hydrogen, C_{1-6}alkyl, C_{2-6}alkenyl or C_{2-7}alkynyl;

R_{13} is independently divalent Ar, C_{1-10}alkylene, C_{1-10}alkylidene, C_{2-10}alkenylene, all of which may be unsubstituted or substituted by one or more OH, CH_2OH, $N(R_6)_2$ or halogen;

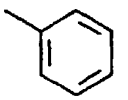
R_{14} is independently hydrogen, C_{1-10}alkyl, XC_{1-10}alkyl, Ar or XAr;

R_{15} is independently C_{1-6}alkyl or phenyl substituted by one or two C_{1-6}alkyl, OH, C_{1-5}alkoxy, $S(O)_qR_6$, $N(R_6)_2$, Br, F, I, Cl, CF_3 or $NHCOR_6$;

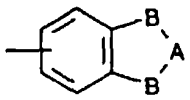
X is independently $(CH_2)_n$, O, NR_6 or $S(O)_q$;

Y is independently CH_3 or $X(CH_2)_nAr$;

Ar is independently :



(a)



(b)

naphthyl, furyl, oxozolyl, indolyl, pyridyl, thienyl, oxazolidinyl, thiazolyl, isothiazolyl, pyrazolyl, triazolyl, tetrazolyl, imidazolyl, imidazolidinyl, thiazolidinyl, isoxazolyl, oxadiazolyl, thiadiazolyl, morpholinyl, piperidinyl, piperazinyl, pyrrolyl, or pyrimidyl; all of which may be unsubstituted or substituted by one or more Z_1 or Z_2 groups;

A is independently C=0, or $(C(R_6)_2)_m$;

B is independently $-CH_2-$ or -0-;

Z_1 and Z_2 are independently hydrogen, XR_6, C_{1-8}alkyl, $(CH_2)_qCO_2R_6$, $C(O)N(R_6)_2$, CN, $(CH_2)_nOH$, NO_2, F, Cl, Br, I, $N(R_6)_2$, $NHC(O)R_6$, $X(CH_2)_nR_8$, $O(CH_2)_mC(O)NR_aSO_2R_{15}$, $(CH_2)_mOC(O)NR_aSO_2R_{15}$, $O(CH_2)_m$ $NR_aC(O)NR_aSO_2R_{15}$, or tetrazolyl which may be substituted or unsubstituted by C_{1-6}alkyl, CF_3 or $C(O)R_6$;

Ar' is naphthyl, furyl, oxozolyl, indolyl, pyridyl, thienyl, oxazolidinyl, thiazolyl, isothiazolyl, pyrazolyl, triazolyl, tetrazolyl, imidazolyl, imidazolidinyl, thiazolidinyl, isoxazolyl, oxadiazolyl, thiadiazolyl, morpholinyl, piperidinyl,

piperazinyl, pyrrolyl, or pyrimidyl; all of which may be unsubstituted or substituted by one or more XR_9-Y, Z_1 or Z_2 groups;

m is independently 1 to 3;
n is independently 0 to 6;
q is independently 0, 1 or 2;
provided R_3, R_4 and R_5 are not O-O$(CH_2)_n$Ar;
or a pharmaceutically acceptable salt thereof.

All alkyl, alkenyl, alkynyl and alkoxy groups may be straight or branched.

The compounds of the present invention may contain one or more asymmetric carbon atoms and may exist in racemic and optically active form. All of these compounds and diastereoisomers are contemplated to be within the scope of the present invention.

The preferred compounds are:

(*E*)-*alpha*-[[5-[4-[(2-carboxyphenyl)methoxy]-2-methoxypyrid-5-yl]isoxazol-4-yl]methylene]-6-methoxy-1,3-benzodioxole-5-propanoic acid;

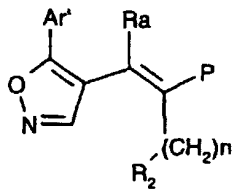
(*E*)-*alpha*-[[5-(3-carboxy-5-chlorothien-2-yl)isoxazol-4-yl]methylene]-6-methoxy-1,3-benzodioxole-5-propanoic acid;

(*E*)-*alpha*-[[3-[4-[(2-carboxyphenyl)methoxy]-2-methoxypyrid-5-yl]isoxazol-4-yl]methylene]-6-methoxy-1,3-benzodioxole-5-propanoic acid;

(*E*)-*alpha*-[[3-(3-carboxy-5-chlorothien-2-yl)isoxazol-4-yl]methylene]-6-methoxy-1,3-benzodioxole-5-propanoic acid;

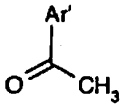
(*E*)-*alpha*-[[3-Butyl-4-[4-[(2-carboxyphenyl)methoxy]-2-methoxypyrid-5-yl]isoxazol-5-yl]methylene]-6-methoxy-1,3-benzodioxole-5-propanoic acid;

(*E*)-*alpha*-[[5-Butyl-4-[4-[(2-carboxyphenyl)methoxy]-2-methoxypyrid-5-yl]isoxazol-3-yl]methylene]-6-methoxy-1,3-benzodioxole-5-propanoic acid;



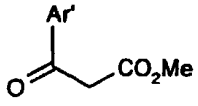
(Id)

Compounds of the Formula (Id), can be prepared starting from a ketone of Formula (2) wherein Ar' is defined as in formula (Id)



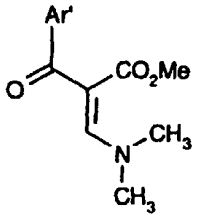
(2)

which is reacted with dimethyl carbonate in the presence of a base such as sodium hydride at 40-70 °C to provide a keto ester of Formula (3).



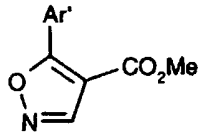
(3)

Reaction of keto ester of Formula (3) with N,N-dimethylformamide dimethyl acetal in hot toluene provides the enamine of Formula (4).

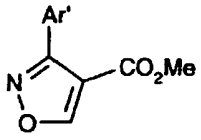


(4)

Treatment of compound of Formula (4) with hydroxylamine hydrochloride ($NH_2OH \cdot HCl$) in a suitable solvent such as either methanol or aqueous methanol and in the presence of a base such as sodium acetate affords a mixture isoxazoles of Formulas (5) and (6), which can be separated by silica gel chromatography or by selective recrystallization.

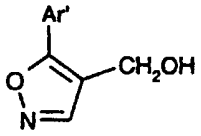


(5)



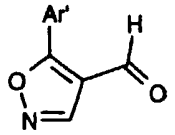
(6)

Reduction of isoxazole of Formula (5) with either diisobutylaluminum hydride in dichloromethane or lithium borohydride in tetrahydrofuran at low temperature produces the alcohol of Formula (7).



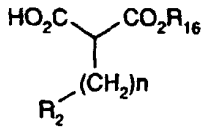
(7)

Oxidation of the primary alcohol of Formula (7) with either Jones reagent at ambient temperature or with manganese dioxide in refluxing toluene affords the aldehyde of Formula (8).



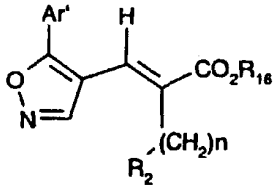
(8)

Knoevenagel condensation of isoxazolyl aldehyde of Formula (8) with a half acid of Formula (9), wherein R_{16} is allyl and R_2 is defined as in formula (Id)



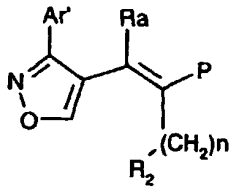
(9)

in a solvent such as benzene at reflux, in the presence of piperidinium acetate with azeotropic removal of water using a Dean-Stark apparatus provids an ester of Formula (10).



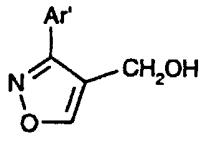
(10)

Deprotection of allyl ester of Formula (10) using triethylsilane in the presence of a catalytic amount of tetrakis(triphenylphosphine)palladium(0) in a suitable solvent such as tetrahydrofuran at reflux affords, after acidification with acetic acid, an acid of the Formula (Id), wherein P is CO_2H.



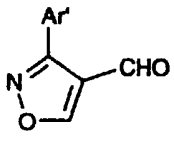
(Ie)

Likewise, compounds of Formula (Ie) can be prepared from isoxazolyl ester of Formula (6) by reduction with either diisobutylaluminum hydride in dichloromethane or lithium borohydride in tetrahydrofuran at low temperature to provide the primary alcohol of Formula (11)



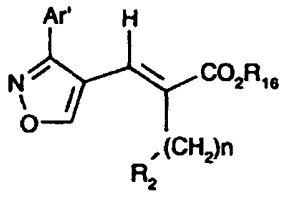
(11)

Oxidation of compound of Formula (11) with Jones reagent at room temperature afforded the aldehyde of Formula (12),



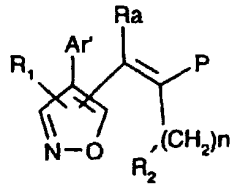
(12)

which was condensed with half acid of Formula (9), in refluxing benzene and in the presence of piperidinium acetate with azeotropic removal of water using a Dean-Stark apparatus, to provide an ester of Formula (13).



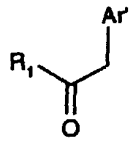
(13)

Deprotection of allyl ester of Formula (13) using triethylsilane in the presence of a catalytic amount of tetrakis(triphenylphosphine)palladium(0) in a suitable solvent such as tetrahydrofuran at reflux affords, after acidification with acetic acid, an acid of the Formula (Ie), wherein P is CO_2H.



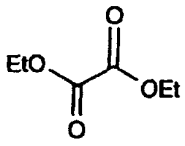
(Ie)

Compounds of Formula (Ie) can be prepared starting by commercially available ketones of Formula (14)



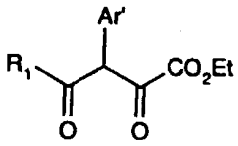
(14)

by reaction with diethyl oxalate of Formula (15)



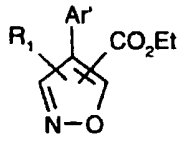
(15)

in the presence of a base such as sodium ethoxide in a solvent such as ethanol to produce a diketone of Formula (16).



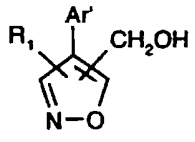
(16)

Reaction of a diketone of Formula (16) with hydroxylamine hydrochloride ($NH_2OH \cdot HCl$) in a suitable solvent such as pyridine at reflux provides an isoxazole of Formula (17)



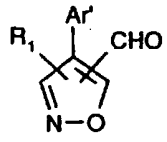
(17)

Reduction of the isoxazole of Formula (17) with either diisobutylaluminum hydride in dichloromethane or lithium borohydride in tetrahydrofuran at low temperature provided the primary alcohol of Formula (18).



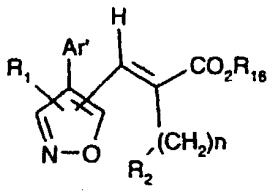
(18)

Oxidation of the isoxazolyl alcohol of Formula (18) with either Jones reagent at ambient temperature or with manganese dioxide in refluxing toluene afforded the aldehyde of Formula (19),



(19)

which was condensed with half acid of Formula (9) in refluxing benzene in the presence of piperidinium acetate with azeotropic removal of water to afford an ester of Formula (20).



(20)

Deprotection of allyl ester of Formula (20) using triethylsilane in the presence of a catalytic amount of tetrakis(triphenylphosphine)palladium(0) in a suitable solvent such as tetrahydrofuran at reflux affords, after acidification with acetic acid, an acid of the Formula (Ie), wherein P is CO_2H and R_a is H.

In order to use a compound of the Formula (I) or a pharmaceutically acceptable salt thereof for the treatment of humans and other mammals it is normally formulated in accordance with standard pharmaceutical practice as a pharmaceutical composition.

Compounds of Formula (I) and their pharmaceutically acceptable salts may be administered in a standard manner for the treatment of the indicated diseases, for example orally, parenterally, sub-lingually, transdermally, rectally, via inhalation or via buccal administration.

Compounds of Formula (I) and their pharmaceutically acceptable salts which are active when given orally can be formulated as syrups, tablets, capsules and lozenges. A syrup formulation will generally consist of a suspension or solution of the compound or salt in a liquid carrier for example, ethanol, peanut oil, olive oil, glycerine or water with a flavouring or colouring agent. Where the composition is in the form of a tablet, any pharmaceutical carrier routinely used for preparing solid formulations may be used. Examples of such carriers include magnesium stearate, terra alba, talc, gelatin, agar, pectin, acacia, stearic acid, starch, lactose and sucrose. Where the composition is in the form of a capsule, any routine encapsulation is suitable, for example using the aforementioned carriers in a hard

gelatin capsule shell. Where the composition is in the form of a soft gelatin shell capsule any pharmaceutical carrier routinely used for preparing dispersions or suspensions may be considered, for example aqueous gums, celluloses, silicates or oils and are incorporated in a soft gelatin capsule shell.

Typical parenteral compositions consist of a solution or suspension of the compound or salt in a sterile aqueous or non-aqueous carrier optionally containing a parenterally acceptable oil, for example polyethylene glycol, polyvinylpyrrolidone, lecithin, arachis oil, or sesame oil.

Typical compositions for inhalation are in the form of a solution, suspension or emulsion that may be administered as a dry powder or in the form of an aerosol using a conventional propellant such as dichlorodifluoromethane or trichlorofluoromethane.

A typical suppository formulation comprises a compound of Formula (1) or a pharmaceutically acceptable salt thereof which is active when administered in this way, with a binding and/or lubricating agent, for example polymeric glycols, gelatins, cocoa-butter or other low melting vegetable waxes or fats or their synthetic analogues.

Typical transdermal formulations comprise a conventional aqueous or non-aqueous vehicle, for example a cream, ointment, lotion or paste or are in the form of a medicated plaster, patch or membrane.

Preferably the composition is in unit dosage form, for example a tablet, capsule or metered aerosol dose, so that the patient may administer to themselves a single dose.

Each dosage unit for oral administration contains suitably from 0.1 mg to 500 mg/Kg, and preferably from 1 mg to 100 mg/Kg, and each dosage unit for parenteral administration contains suitably from 0.1 mg to 100 mg, of a compound of Formula (I) or a pharmaceutically acceptable salt thereof calculated as the free acid. Each dosage unit for intranasal administration contains suitably 1-400 mg and preferably 10 to 200 mg per person. A topical formulation contains suitably 0.01 to 1.0% of a compound of Formula (I).

The daily dosage regimen for oral administration is suitably about 0.01 mg/Kg to 40 mg/Kg, of a compound of Formula (I) or a pharmaceutically acceptable salt thereof calculated as the free acid. The daily dosage regimen for parenteral administration is suitably about 0.001 mg/Kg to 40 mg/Kg, of a compound of the Formula (I) or a pharmaceutically acceptable salt thereof calculated as the free acid. The daily dosage regimen for intranasal administration and oral inhalation is suitably about 10 to about 500 mg/person. The active ingredient may be administered from 1 to 6 times a day, sufficient to exhibit the desired activity.

No unacceptable toxicological effects are expected when compounds of the invention are administered in accordance with the present invention.

The biological activity of the compounds of Formula (I) are demonstrated by the following tests:

I. Binding Assay

A) CHO cell membrane preparation.

CHO cells stably transfected with human ET_A and ET_B receptors were grown in 245 mm x 245 mm tissue culture plates in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. The confluent cells were washed with Dulbecco's phosphate-buffered saline containing a protease inhibitor cocktail (5 mM EDTA, 0.5 mM PMSF, 5 ug/ml of leupeptin and 0.1 U/ml of aprotinin) and scraped in the same buffer. After centrifugation at 800 *x g*, the cells were lysed by freezing in liquid nitrogen and thawing on ice followed by homogenization (30 times using a glass dounce homogenizer) in lysis buffer containing 20 mM Tris HCl, pH 7.5, and the protease inhibitor cocktail. After an initial centrifugation at 800 x *g* for 10 min to remove unbroken cells and nuclei, the supernatants were centrifuged at 40,000 *x g* for 15 min and the pellet was resuspended in 50 mM Tris HCl, pH 7.5, and 10 mM $MgCl_2$ and stored in small aliquots at -70°C after freezing in liquid N_2. Protein was determined by using the BCA method and BSA as the standard.

(B) Binding studies.

[^{125}I]ET-1 binding to membranes prepared from CHO cells was performed following the procedure of Elshourbagy *et al.* (1993). Briefly, the assay was initiated in a 100 ul volume by adding 25 ul of [^{125}I]ET-1 (0.2-0.3 nM) in 0.05% BSA to membranes in the absence (total binding) or presence (nonspecific binding) of 100 nM unlabeled ET-1. The concentrations of membrane proteins were 0.5 and 0.05 ug per assay tube for ET_A and ET_B receptors, respectively. The incubations (30°C, 60 min) were stopped by dilution with cold buffer (20 mM Tris HCl, pH 7.6, and 10 mM $MgCl_2$) and filtering through Whatman GF/C filters (Clifton, NJ) presoaked in 0.1% BSA. The filters were washed 3 times (5 ml each time) with the same buffer by using a Brandel cell harvester and were counted by using a gamma counter at 75% efficiency.

The following example is illustrative and are not limiting of the compounds of this invention.

EXAMPLE 1

Formulations for pharmaceutical use incorporating compounds of the present invention can be prepared in various forms and with numerous excipients. Examples of such formulations are given below.

Inhalant Formulation

A compound of Formula I, (1 mg to 100 mg) is aerosolized from a metered dose inhaler to deliver the desired amount of drug per use.

Tablets/Ingredients		Per Tablet
1.	Active ingredient (Cpd of Form. I)	40 mg
2.	Corn Starch	20 mg
3.	Alginic acid	20 mg

4.	Sodium Alginate	20 mg
5.	Mg stearate	1.3 mg
		2.3 mg

Procedure for tablets:

Step 1 Blend ingredients No. 1, No. 2, No. 3 and No. 4 in a suitable mixer/blender.

Step 2 Add sufficient water portion-wise to the blend from Step 1 with careful mixing after each addition. Such additions of water and mixing until the mass is of a consistency to permit its conversion to wet granules.

Step 3 The wet mass is converted to granules by passing it through an oscillating granulator using a No. 8 mesh (2.38 mm) screen.

Step 4 The wet granules are then dried in an oven at 140°F (60°C) until dry.

Step 5 The dry granules are lubricated with ingredient No. 5.

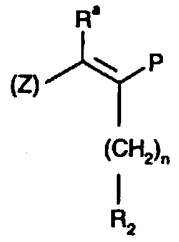
Step 6 The lubricated granules are compressed on a suitable tablet press.

Parenteral Formulation

A pharmaceutical composition for parenteral administration is prepared by dissolving an appropriate amount of a compound of formula I in polyethylene glycol with heating. This solution is then diluted with water for injections Ph Eur. (to 100 ml). The solution is then steriled by filtration through a 0.22 micron membrane filter and sealed in sterile containers.

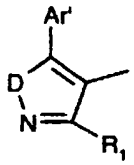
CLAIMS:

1. A compound of Formula (I):

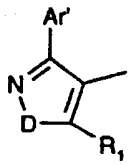


(I)

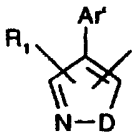
wherein Z is



(d)

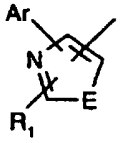


(e)



(f)

or



(g)

D is O or S;

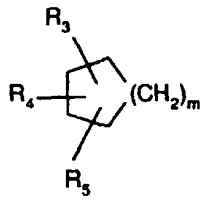
E is O, S or NR_{15};

P is tetrazol-5-yl, CO_2R_6 or $C(O)N(R_6)S(O)_qR_{10}$;

R^a is hydrogen or C_{1-6}alkyl;

R_1 is independently hydrogen, Ar or C_{1-6}alkyl;

R_2 is Ar, C_{1-8}alkyl, $C(O)R_{14}$ or



(c)

R_3 and R_5 are independently R_{13} OH, C_{1-8}alkoxy, $S(O)_qR_{11}$, $N(R_6)_2$, Br, F, I, Cl, CF_3, $NHCOR_6$ $R_{13}CO_2R_7$, -X-R_9-Y or $-X(CH_2)_nR_8$ wherein each methylene group within $-X(CH_2)_nR_8$ may be unsubstituted or substituted by one or two $-(CH_2)_nAr$ groups;

R_4 is independently R_{11}, OH, C_{1-5}alkoxy, $S(O)_qR_{11}$, $N(R_6)_2$, Br, F, I, Cl or $NHCOR_6$, wherein the C_{1-5}alkoxy may be unsubstituted or substituted by OH, methoxy or halogen;

R_6 is independently hydrogen or C_{1-8}alkyl;

R_7 is independently hydrogen, C_{1-10}alkyl, C_{2-10}alkenyl or C_{2-8}alkynyl, all of which may be unsubstituted or substituted by one or more OH, $N(R_6)_2$, CO_2R_{12}, halogen or XC_{1-10}alkyl; or R_7 is $(CH_2)_nAr$;

R_8 is independently R_{11}, CO_2R_7, $CO_2C(R_{11})_2O(CO)XR_7$, $PO_3(R_7)_2$, $SO_2NR_7R_{11}$, $NR_7SO_2R_{11}$, $CONR_7SO_2R_{11}$, SO_3R_7, SO_2R_7, $P(O)(OR_7)R_7$, CN, $CO_2(CH_2)_mC(O)N(R_6)_2$, $C(R_{11})_2N(R_7)_2$, $C(O)N(R_6)_2$, $NR_7C(O)NR_7SO_2R_{11}$, tetrazole or OR_6;

R_9 is independently a bond, C_{1-10}alkylene, C_{1-10}alkenylene, C_{1-10}alkylidene, C_{1-10}alkynylene, all of which may be linear or branched, or phenylene, all of which may be unsubstituted or substituted by one of more OH, $N(R_6)_2$, COOH or halogen;

R_{10} is independently C_{1-10}alkyl, $N(R_6)_2$ or Ar;

R_{11} is independently hydrogen, Ar, C_{1-8}alkyl, C_{2-8}alkenyl, C_{2-8}alkynyl, all of which may be unsubstituted or substituted by one or more OH, CH_2OH, $N(R_6)_2$ or halogen;

R_{12} is independently hydrogen, C_{1-6}alkyl, C_{2-6}alkenyl or C_{2-7}alkynyl;

R_{13} is independently divalent Ar, C_{1-10}alkylene, C_{1-10}alkylidene, C_{2-10}alkenylene, all of which may be unsubstituted or substituted by one or more OH, CH_2OH, $N(R_6)_2$ or halogen;

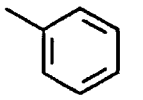
R_{14} is independently hydrogen, C_{1-10}alkyl, XC_{1-10}alkyl, Ar or XAr;

R_{15} is independently C_{1-6}alkyl or phenyl substituted by one or two C_{1-6}alkyl, OH, C_{1-5}alkoxy, $S(O)_qR_6$, $N(R_6)_2$, Br, F, I, Cl, CF_3 or $NHCOR_6$;

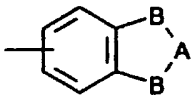
X is independently $(CH_2)_n$, O, NR_6 or $S(O)_q$;

Y is independently CH_3 or $X(CH_2)_nAr$;

Ar is independently :



(a)



(b)

naphthyl, furyl, oxozolyl, indolyl, pyridyl, thienyl, oxazolidinyl, thiazolyl, isothiazolyl, pyrazolyl, triazolyl, tetrazolyl, imidazolyl, imidazolidinyl, thiazolidinyl, isoxazolyl, oxadiazolyl, thiadiazolyl, morpholinyl, piperidinyl, piperazinyl, pyrrolyl, or pyrimidyl; all of which may be unsubstituted or substituted by one or more Z_1 or Z_2 groups;

A is independently C=0, or $(C(R_6)_2)_m$;

B is independently $-CH_2-$ or -0-;

Z_1 and Z_2 are independently hydrogen, XR_6, C_{1-8}alkyl, $(CH_2)_qCO_2R_6$, $C(O)N(R_6)_2$, CN, $(CH_2)_nOH$, NO_2, F, Cl, Br, I, $N(R_6)_2$, $NHC(O)R_6$, $X(CH_2)_nR_8$, $O(CH_2)_mC(O)NR_aSO_2R_{15}$, $(CH_2)_mOC(O)NR_aSO_2R_{15}$, $O(CH_2)_m$ $NR_aC(O)NR_aSO_2R_{15}$, or tetrazolyl which may be substituted or unsubstituted by C_{1-6}alkyl, CF_3 or $C(O)R_6$;

Ar' is naphthyl, furyl, oxozolyl, indolyl, pyridyl, thienyl, oxazolidinyl, thiazolyl, isothiazolyl, pyrazolyl, triazolyl, tetrazolyl, imidazolyl, imidazolidinyl, thiazolidinyl, isoxazolyl, oxadiazolyl, thiadiazolyl, morpholinyl, piperidinyl, piperazinyl, pyrrolyl, or pyrimidyl; all of which may be unsubstituted or substituted by one or more XR_9-Y, Z_1 or Z_2 groups;

m is independently 1 to 3;

n is independently 0 to 6;

q is independently 0, 1 or 2;

provided R_3, R_4 and R_5 are not O-$O(CH_2)_nAr$;

or a pharmaceutically acceptable salt thereof.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/00955

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07D 409/14
US CL :548/247

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 548/247

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 3,277,105 A (SCHMIDT et al) 04 October 1966 (04/10/66), see entire document, especially column 1.	1,part
A	US 3,700,661 A (SAUCY et al) 24 October 1972 (24/10/72), see entire document, especailly column 2 formula F.	1,part

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 21 MARCH 1997	Date of mailing of the international search report 09 JUN 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer ROBERT W. RAMSUER Telephone No. (703) -308-1235

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/00955

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 1(part)
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Please See Extra Sheet.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/00955

BOX I. OBSERVATIONS WHERE CLAIMS WERE FOUND UNSEARCHABLE

2. Where no meaningful search could be carried out, specifically:

The multitude of variables and their permutations and combinations (e.g.Z,Ar,D,E,P,R1,R2,R3,R4,X,Y,the proviso,etc.) result in claimed subject matter that is so broad in scope that it is rendered virtually incomprehensible and thus no meaningful search can be given. Note also that the claimed subject matter lacks a significant structural element qualifying as the special technical feature that clearly defines a contribution over the art. The subject matter claimed contains a C=C group which does not define a contribution over the prior art, Therefore, the first descernable invention as found on page 8 line 16(the compound thereat) has been searched.